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GENETIC STOCK

Registration of A/BN641 and RN642 *waxy* Grain Sorghum Genetic Stocks

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ABSTRACT

Loss-of-function mutations in the *granule-bound starch synthase* gene result in an endosperm with a waxy appearance and a near absence of starch amylose. Three *waxy* grain sorghum [*Sorghum bicolor* (L.) Moench] lines, AN641 (Reg. No. GS-741, PI 672150), BN641 (Reg. No. GS-742, PI 672151), and RN642 (Reg. No. GS-743, PI 672152) were developed jointly by the USDA–ARS and the Agricultural Research Division, Institute of Agriculture and Natural Resources, University of Nebraska, and were released in June 2014. AN641 and BN641 have the *waxy*^{b} (*wx*^{b}) allele and are near-isogenic to 'Wheatland'. RN642 has the *waxy*^{a} (*wx*^{a}) allele and is near-isogenic to 'Tx430'. Release of these lines with cytoplasmic male-sterile (A), maintainer (B), and fertility restorer (R) fertility reactions to A$_1$ cytoplasm facilitates the production and evaluation of interallelic (*wx*^{b} × *wx*^{a}) *waxy* and hetero*waxy* [*wx*^{b} × wild-type (WT) and WT × *wx*^{a}] hybrids as a source of low-amylose starch for the ethanol and food industries.

In grain ethanol production, starch amylose increases viscosity and forms complexes with lipids, which restrict access of hydrolytic enzymes to starch molecules and lengthen fermentation times (Sharma et al., 2007; Wang et al., 2008; Wu et al., 2008; Wu et al., 2010; Yan et al., 2011). The food industry uses low-amylose starch to reduce the viscosity of pastes and to increase the shelf life of breads, cakes, and pastes (Wang and Copeland, 2013). Additional sources of starch with a range of amylose contents are sought for customized product development, to alter the glycemic index of food, or to confer desired rheological properties (Sang et al., 2008; Zhu, 2014). Common sources of low-amylose starch include *waxy* corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and potato (*Solanum tuberosum* L.). Sorghum [*Sorghum bicolor* (L.) Moench] originated in northeast Africa and is adapted to semiarid environments. Development of grain sorghum parent lines suitable for *waxy* and hetero*waxy* (having one *waxy* and one wild-type [WT] allele) sorghum hybrid production provides an opportunity to produce low-amylose starch in dryland and irrigated cropping systems where decreased water use is desirable.

Amylose is synthesized by granule-bound starch synthase (GBSS) (Denyer et al., 2001), encoded by the *Wx* gene (Sb10 g002140) in sorghum. In sorghum, two loss-of-function mutations in *Wx* were identified and characterized (Pedersen et al., 2005; Sattler et al., 2009). The *wx*^{a} allele contained a large DNA insertion within the third exon, and both GBSS protein and enzyme activity were undetectable in the grain (Pedersen et al., 2005; Sattler et al., 2009). The *wx*^{b} allele contained a missense mutation, resulting in substitution of a histidine for glutamine at amino acid 268. The GBSS protein was present, but enzyme activity was reduced by >75% in *wx*^{b} grain relative to wild-type (Pedersen et al., 2005; Sattler et al., 2009). Thus, while amylose content of WT grain ranges from 20 to 50%, amylose content of *waxy* grain ranges from 0 to 5% (Rooney and Serna-Saldivar, 2000; Zhu, 2014). Hetero*waxy* grain has intermediate amylose

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Abbreviations: GBSS, granule-bound starch synthase; WT, wild-type.

content, depending on the dose of *wx* alleles in the triploid endosperm (Ring et al., 1989).

The USEPA announced that ethanol produced from sorghum grain qualifies as an advanced biofuel (USEPA, 2012), which has increased the ethanol industry's interest in sorghum. Despite the benefits of low-amylose starch for the ethanol and food industries, *wx* hybrids historically have been associated with yield drag relative to WT hybrids (Rooney et al., 2005). Using two different *wx* alleles in a hybrid background should minimize the effects of recessive deleterious genes linked to the *Wx* locus. Commercial hybrid sorghum seed production requires the use of cytoplasmic male-sterile (A) lines, maintainer (B) lines, and restorer (R) lines, capable of restoring male fertility in F_1 hybrids. The wx^b and wx^a alleles were introgressed into sorghum cultivars Wheatland and Tx430, respectively, to develop A, B and R lines—AN641 (Reg. No. GS-741, PI 672150), BN641 (Reg. No. GS-742, PI 672151), and RN642 (Reg. No. GS-743, PI 672152)—that will facilitate production and evaluation of *waxy* and hetero*waxy* hybrids.

Materials and Methods

The *waxy* sorghum lines near-isogenic to Wheatland (Brown et al., 1936) and Tx430 (Miller 1984) were developed through the introgression of *wx* alleles into genetically (nuclear) *male-sterile 3* (*ms3*) versions of BWheatland and RTx430 (Pedersen et al., 1997) by crossing with *waxy* lines BTxARG-1 (wx^b) and RTx2907 (wx^a) (Pedersen et al., 2005), respectively. F_1 progeny of the *ms3* BWheatland × BTxARG-1 (wx^b) cross were self-pollinated. F_2 progeny homozygous for *ms3* and segregating for *wx* were backcrossed with WT BWheatland. The BC_1 progeny was self-pollinated, and BC_1/S_1 individuals homozygous for *ms3* and segregating for *wx* were backcrossed with WT BWheatland. A total of four backcrosses were made in this manner. The BC_4/S_2 was screened for *wx Ms3Ms3* progeny, which were used in paired crosses for six generations to develop AN641 and BN641 as follows. The BC_4/S_2 was crossed to the A_1 cytoplasm source AWheatland to generate AWheatland × BC_4/S_2 progeny (the AN641 experimental line) and simultaneously self-pollinated to generate the BC_4/S_3 (the BN641 experimental line). The BN641 experimental line was crossed to the AN641 experimental line and subsequently self-pollinated until the BC_4/S_8 generation, when the finished AN641 and BN641 line (synonym = wx^b A/BWheatland) was released. Similar methods used to develop BN641 were used to develop progeny from the *ms3* RTx430 × RTx2907 (wx^a) cross into RN642 (synonym = wx^a RTx430). Pedigree information for AN641, BN641 and RN642 is presented in Table 1. Allele-specific primers (Sattler et al., 2009) and iodine staining (Pedersen et al., 2004) were used to verify that A/BN641 and RN642 are homozygous for their respective *wx* alleles.

BN641 and RN642 were evaluated for agronomic performance and yield on a per se basis in field trials at Mead, NE, in 2007 and 2008 (Sharpsburg silty clay loam; fine smectitic, mesic Typic Agriudoll); and at Lincoln, NE, in 2008 (Kennebec silt loam; fine-silty, mixed, superactive, mesic Cumulic Hapludoll) (three environments total). Wild-type BWheatland and RTx430 were included for comparison. The lines (four entries total) were sown at 120 seeds per row (240,000 seeds ha^{-1}) delivered by a precision vacuum planter in the third week of May. Plots consisted of two 6.6-m rows spaced 76 cm apart. For all locations, nitrogen fertilizer was applied at 112 kg ha^{-1} before planting. Atrazine [6-chloro-*n*-ethyl-*N′*(1-methylethyl)-1,3,5-triazine-2,4,diamine] was applied at 1.12 kg ha^{-1} immediately after planting, followed by an application of quinclorac (3,7-dichloro-8-quinolinecarboxylic acid) and atrazine at 0.37 kg ha^{-1} and 0.56 kg ha^{-1}, respectively, 4 wk after planting at Mead 2007. Atrazine (0.37 kg ha^{-1}) and alachlor [2-Chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl) acetamide] were applied at 1.25 kg a.i. ha^{-1} 2 wk after planting at Mead 2008. Supplemental irrigation (3.8 cm) was applied at Mead via overhead sprinklers on 29 June, 5 July, 16 July, and 26 July 2007. Irrigation (2.5 cm) was applied on 30 July and 5 September at Mead in 2008; 3.8 cm was applied 15 August. No supplemental irrigation was applied at Lincoln. Baythroid [Cyano(4-fluoro-3-phenoxyphenyl)methyl-3-(2,2-dichloro-ethenyl)-2,2-dimethyl-cyclopropanecarboxylate] was applied at 0.3 kg a.i. ha^{-1} on 1 August at Mead 2008 to control for grasshoppers (Orthoptera).

The experimental design was a randomized complete block with four replicates. Agronomic characters recorded included field emergence (plants m^{-1}) approximately 4 wk post-planting, days to 50% anthesis, and height (cm) at the top of the mature panicle. Grain was hand-harvested and air-dried to constant mass, threshed, weighed, and adjusted to 145 g kg^{-1} moisture before yield analysis (kg ha^{-1}). Test weight (kg hL^{-1}) also was recorded. Data were subjected to analysis of variance in PROC MIXED of SAS version 9.2 (SAS Institute, 2012) to generate least squares (LS) means and *P* values. Entry was considered a fixed effect and environment, replicate within environment, and environment × entry were considered random in determining the effect of entry on the response variables measuring agronomic performance and yield. LS means were separated at $P = 0.05$ using Fisher's Protected LSD test.

Characteristics

Complete comparisons of agronomic performance are presented in Table 2. BN641 resembles BWheatland, as it has a purple necrotic lesion color, no awns, a red caryopsis, juicy culms, and no tannins in the grain, and it does not restore fertility in A_1 cytoplasm. Averaged over environments, BN641 field emergence, days to 50% anthesis, and test weight were

Table 1. Pedigrees, fertility reactions, and endosperm type of *waxy* sorghum genetic stocks.

Entry	Synonym	Pedigree	Fertility reaction†	Endosperm
AN641	wx^b AWheatland	BC_6 [AWheatland × wx^b BWheatland]	A	waxy
BN641	wx^b BWheatland	S_8 [(F_2 *ms3* BWheatland × B TxARG-1 (wx^b))/BWheatland BC_4]	B	waxy
RN642	wx^a RTx430	S_5 [(F_2 *ms3* RTx430 × RTx2907 (wx^a))/RTx430 BC_4]	R	waxy

† Fertility reaction to A_1 cytoplasmic male-sterile cytoplasm: A/B = male-sterile/maintainer pair, R = fertility restorer.

Table 2. Entry, agronomic performance, and yield of near-isogenic *waxy* and wild-type sorghum genetic stocks. Data were pooled over three environments: Mead, NE, in 2007 and 2008; and Lincoln, NE, in 2007.

Entry	Endosperm	Emergence	50% anthesis	Height	Yield‡	Test weight
				Mean†		
		plants m^{-1}	d	cm	kg ha^{-1}	kg hL^{-1}
B-lines						
BWheatland	wild-type	20 a	76 a	109 a	6200 a	66 a
BN641	*waxy*	20 a	74 a	120 b	6942 b	69 a
R-lines						
RTx430	wild-type	20 a	71 a	130 a	6702 a	60 a
RN642	*waxy*	20 a	73 a	138 b	6717 a	59 a
$LSD_{0.05}$		2.3	5.1	4.2	621	4.6

† Means with the same letter within columns and B or R fertility reactions do not differ at $P = 0.05$.

‡ Grain yield was adjusted to 145 g kg^{-1} moisture before analysis.

similar to BWheatland, but BN641 was taller (120 cm vs. 109 cm, $P < 0.01$), and yielded more grain (6942 kg ha^{-1} vs. 6200 kg ha^{-1}, $P = 0.02$) than BWheatland.

RN642 resembles RTx430, as it has purple necrotic lesion color, no awns, a translucent white caryopsis, yellow endosperm, juicy culms, and no tannins in the grain, and it restores fertility in A_1 cytoplasm. RN642 field emergence, days to 50% anthesis, grain yield, and test weight were similar to RTx430, but RN642 was taller (138 cm vs. 130 cm, $P < 0.01$) than RTx430.

The *waxy* trait in sorghum lines and hybrids has been associated with yield drag (Rooney et al., 2005), which Jampala et al. (2012) suggested could be overcome by focusing breeding efforts to eliminate deleterious alleles linked to the *Wx* locus. Yield of BN641 was greater than that of BWheatland, and yield of RN642 did not differ statistically from RTx430; thus, both the wx^a and wx^b alleles were not associated with reduced yield in their respective lines. It should be noted that BN641 and RN642 were taller than the WT. The combining abilities of AN641 and RN642 are currently unknown.

AN641, BN641 and RN642 are three-dwarf, combine-height, photoperiod-insensitive lines with similar agronomic performance, flowering time, and grain yield to Wheatland and Tx430, respectively. These traits are desirable for commercial production of *waxy* and hetero*waxy* grain sorghum hybrids in temperate regions of the world. The drought tolerance of sorghum in particular may be used to produce low-amylose starch in both dryland and irrigated systems where reduced water use is desired. A/B and R fertility reactions facilitate crossing with each other or with additional *waxy* and WT lines of similar maturity (71 to 76 d) to breed for local adaptation and to expand sources of low-amylose starch for the ethanol and food industries.

Conclusions

Release of A/BN641 and RN642 makes available two different *wx* alleles of sorghum, wx^a and wx^b, with A/B and R fertility reactions, respectively. Neither line demonstrated reduced field emergence or yield relative to their corresponding parental line in field trials in three environments. Each may be crossed with one another or with other *waxy* or WT lines to generate *waxy* and hetero*waxy* hybrids useful for the production of low-amylose starch.

Availability

Seed of these sorghum genetic stocks will be maintained and distributed by the USDA–ARS, Grain, Forage, and Bioenergy Research Unit, Department of Agronomy, University of Nebraska, Lincoln, Nebraska 68583-0937, and will be provided without cost to each applicant on written request. Genetic material of this release has been deposited in the National Plant Germplasm System, where it will be immediately available for research purposes, including development and commercialization of new cultivars. It is requested that appropriate recognition be made if this germplasm contributes to the development of new breeding materials.

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